

Water Absorption of Plastics Molded from Acylated Casein

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CASEIN plastics have been made commercially for many years, but they constitute only a small percentage of the total production of plastics in this country. Among the reasons for this may be cited the relatively high water absorption of the protein and the involved process of manufacture. Treating casein plastics with formaldehyde for considerable periods of time increases their water resistance, but they still exhibit dimensional variations caused by water exchange. Further improvement with respect to water absorption has been sought by chemical modification of the protein prior to molding, and numerous attempts have been made to circumvent the time-consuming hardening treatment with formaldehyde by the use of other reagents. However, the results of these investigations have not been successful enough to find widespread commercial application (23).

The present paper deals principally with the modification of casein by acetic, propionic, and *n*-butyric anhydrides to produce derivatives which can be molded directly into articles having greater resistance to water. Since acetylation decreases the hygroscopicity of cellulose (21), it appeared likely that acetylation of casein would result in a product with reduced affinity for water, in spite of the difference in reactive groups concerned.

Injection of propionyl and butyryl groups into cellulose has received considerable attention, but the writers are not aware of any previous attempt to modify casein in a similar manner. Such modification, if successful, should decrease the water absorption of casein more markedly than would acetylation.

One other type of casein modification is described in the present report—namely, acylation and alkylation together. This reaction was applied by Blackburn, Middlebrook, and Phillips (1) to wool, silk, and collagen by treating the proteins with a mixture of acetic anhydride and methanol. Although protein was not alkylated in this manner to any large extent, the reaction seemed of sufficient interest to try it with casein.

The literature on acetylation of protein fibers was reviewed recently (3). In addition to the work on fibers, a number of studies have been reported on acetylation of proteins, including one on development of products for commercial application. A brief survey of the various reagents and conditions employed in these investigations follows.

Acetylated zein products prepared by the action of acetic anhydride and ketene on the protein have been claimed to be suitable for films and coatings (4). Landsteiner (12, 13) treated horse-serum proteins, edestin, and gelatin with acetic anhydride and isolated products of different acetyl contents. Troensegaard and co-workers (24, 25) acetylated blood proteins by heating them with acetyl chloride and glacial acetic acid. Fodor and Epstein (5) found that boiling gelatin with acetic anhydride produced degradation products. Haurowitz (7) acetylated egg albumin with acetic anhydride at 100° C. and obtained a derivative containing 3.7% acetyl. Hendrix and Paquin (8) prepared acetylated casein, egg albumin, and edestin by treating the proteins with acetic anhydride at room temperature. The acetylated proteins had greatly diminished acid-combining power, an indication that the basic groups had been covered. Other in-

TABLE II. WATER ABSORPTION OF ACETYLATED AND ETHYLATED CASEIN

Sample No.	Ratio of Ethanol to Acetic Anhydride	Acetyl Content, %	Ethoxyl Content, %	A.S.T.M. 24-Hr. Water Absorption of Disks ^a , %				
				Unhardened	Formaldehyde-hardened ^b	Reacetylated Samples		
						A.S.T.M. 24-Hr. Water Absorption of Disks ^a , %		
						Acetyl Content, %	Unhardened	Formaldehyde-hardened ^c
9	No ethanol	6.3	...	15.8	8.2	10.0	10.3	4.8
10	0.2	6.8	0.1	11.0	7.6	9.7	...	5.3
11	0.4	5.5	1.0	12.6	7.3	9.7	8.3	4.1
12	0.6	4.4	1.1	12.8	7.6	9.5	...	4.8
13	0.8	4.4	1.0	11.7	8.0	9.6	7.8	4.5
14	1.0	3.4	1.1	...	8.4	9.9	...	4.8
15	1.2	3.4	1.2	15.7	8.3	10.1	...	4.6
16	1.4	3.4	1.2	...	8.4	9.8	9.2	4.2

^a Approximate saturation water content values not determined; 72-hr. water absorption figures, obtained by A.S.T.M. procedure, are considerably higher than those shown above but show the same trend.

^b The formaldehyde content of these samples was 0.7% \pm 0.2%; hardening with formaldehyde did not alter appreciably the acetyl content.

^c The formaldehyde content of these samples was 0.6 to 0.7%; the acetyl content ranged from 9.1 to 9.9%, the values averaging 0.3% lower than before hardening with formaldehyde.

employed; under the more drastic conditions of acetylation with samples 5, 6, and 7, the yields dropped to 92–98%. Treatment with formaldehyde was carried out with substantially no change in yields.

In the molding, all these derivatives flowed fairly well in the presence of 12% water and decidedly better than did formaldehyde-hardened unacetylated casein. It was evident, however, that with progressive acetylation the efficacy of water as plasticizer diminished. Except for mildly acetylated sample 1, the products yielded transparent disks ranging in color from yellowish to light brown, depending on the temperature of acetylation. The more highly acetylated the product, the greater the tendency to stick to the mold and to yield brittle disks. These undesirable features, which were particularly pronounced in samples 5, 6, and 7, may perhaps be attributed to the high temperature or to the longer periods of heating used in the acetylation rather than to the small additional increments of acetyl.

Results of the water absorption tests demonstrate that there was a marked reduction in water absorption with progressive acetylation. At the same time, because of the brittleness of the most highly acetylated materials, maximal acetylation was not entirely beneficial. Therefore, the best product in this series was casein acetylated at 110° C. for 2 hours and then hardened with formaldehyde. The effect of formaldehyde will be discussed in greater detail in the next section.

Because of the brittleness and relative weakness of these plastics, induced perhaps by high temperature or long heating, we attempted to achieve maximal acetylation under milder conditions by the use of catalysts with acetic anhydride. Of the possible catalysts investigated, acetic acid and sulfuric acid were not beneficial; sodium acetate and pyridine did promote acetylation but also resulted in the production of inferior molding powders with relatively high water absorptions.

Since casein derivatives containing as much as 10% acetyl have not been described by other investigators, a few comments may be in order concerning the nature and extent of acetylation attained in these experiments. Hendrix and Paquin (8) observed that their acetylated casein, containing 5.32 to 5.88% acetyl, when treated with cold dilute alkali lost fairly definite amounts of acetyl, presumably oxygen-acetyl groups. The residual acetyl groups, bound by the basic groups of the protein, accounted for 2.51 to 3.65% of the alkali-treated acetyl casein. In similar partition experiments with two samples of highly acetylated casein, casein containing 9.0% acetyl yielded 5.6% oxygen-acetyl and retained 3.4% nitrogen-acetyl groups; casein of 9.9% acetyl content gave 6.0% oxygen-acetyl and 3.9% nitrogen-acetyl. These results are in essential agreement with those of Hendrix and Paquin and may be similarly interpreted. Our data permit the further conclusion, however, that the more

highly acetylated the casein, the greater is the proportion of acetyl residues attached to groups other than the basic groups in the protein molecule.

Possible degradation of the casein molecule during acetylation remains to be considered. We have no experimental evidence bearing on this point other than the materially decreased yields noted for samples 5, 6, and 7, but undoubtedly some degradation occurred under the rather drastic conditions employed for these preparations (compare reference 5). Further speculation concerning the theoretical aspects of the acetylation is unwarranted, since our experiments were primarily designed to evaluate it as a means of curtailing the water absorption of casein plastics.

ACETYLATED AND ETHYLATED CASEIN

The experiments of Blackburn, Middlebrook, and Phillips (1) suggested that casein could be alkylated as well as acetylated in a single reaction. It was thought that this might curtail the water absorption of the protein further than by acetylation alone. To investigate this possibility, 100-gram batches of air-dried casein were treated with 430 ml. of absolute ethanol-acetic anhydride mixtures in which the ethanol-acetic anhydride ratios in equivalents shown in Table II were used. Each sample was heated under reflux at 82–85° C. in an oil bath for 4 hours. Vigorous reactions ensued in all the mixtures (except the preparation in which no alcohol was present) with the formation of ethyl acetate. At the end of the heating periods the samples were washed and dried as described previously. Approximate yields of 105 to 108 grams were obtained. Five to eight grams of each sample were reserved for exhaustive extraction and subsequent analysis for acetyl and ethoxyl. Of the remaining 100 grams, one fourth was molded into test disks. One fourth was treated with formaldehyde, and the product, after analysis for acetyl and for formaldehyde, was also molded into disks. The 50-gram portions which remained were further modified as described below.

Increasing the ethanol-acetic anhydride ratio above 0.4 did not materially augment the ethoxyl content of the derivative. The maximum amount introduced, 1.2%, did not have a pronounced effect on water absorption, although strict comparison is not possible because of the different percentages of acetyl in the products. Hardening with formaldehyde, which was accomplished with little or no loss of acetyl, resulted in substantial reduction of the water uptake. The variations in formaldehyde content (Table I, footnote b) are not particularly significant because of the difficulties attendant on washing the precipitates free of excess formaldehyde from the hardening solution (17). However, it is certain that a considerable proportion of the formaldehyde-combining power of casein is lost through acetylation, since unacetylated casein, hardened, washed, and analyzed in the same way as these derivatives, contained 2.8% formaldehyde.

To obtain a more exact evaluation of the effect of the ethoxyl groups on water absorption, each of the remaining 50-gram portions mentioned above was acetylated further with 210 ml. of acetic anhydride at 110° C. for 2 hours. Control experiments had established the fact that such treatment of the derivatives could be carried out without loss of ethoxyl. The mixtures were filtered and washed as before; the yields of air-dried prod-

TABLE V. EFFECT OF ACYLATION ON WATER ABSORPTION OF CASEIN^a

Type of Casein	Approx. Satn. Water Content of Disks, %
Casein	30
Acetylated (10% acetyl)	22
Propionylated (13% propionyl)	16
Butyrylated (14.9% butyryl)	13

^a All samples were hardened with formaldehyde prior to molding.

tempts to accelerate acylation by such catalysts as butyric acid, acetic anhydride, sulfuric acid, and magnesium perchlorate were not successful.

Even though there was probably some degradation of casein in the preparation of these samples, the water-absorption data again demonstrate the pronounced effect of acylation in lowering the uptake of water by the protein. In fact, although maximal incorporation of 15.5% butyryl was not quite attained, some of these derivatives had the lowest water absorption of any of the products described in this report. Also evident was the progressively decreased effect of formaldehyde in curtailing water absorption as the latter was reduced by increasing substitution with butyryl groups.

The molding properties of the highly acylated products in the butyryl series did not differ greatly from those of highly acetylated or propionylated caseins. The molded specimens were brittle and weak. These defects were particularly marked in samples 35, 37, and 38, and may perhaps be attributed to the rather severe conditions required for the introduction of butyryl groups as well as to the fact that water is a poor plasticizer for these derivatives. We have been unable thus far to find a suitable water-insoluble plasticizer for acylated casein.

DISCUSSION OF RESULTS

The data show that the water absorption of casein is decreased progressively by increasing acylation with acetic, propionic, or butyric anhydrides. Comparison of the approximate saturation water contents of the most highly acylated derivatives, containing roughly equivalent numbers of substituent groups and treated with formaldehyde (Table V), permits the further conclusion that there is a stepwise decrease in water uptake with increasing size of acyl groups introduced into casein. Therefore, acylation of casein by higher fatty acid residues might be expected to have even more profound effects on the water absorption of the protein.

The water absorption of commercial casein plastics as determined by the A.S.T.M. method is 7 to 14% in 24 hours. Thus the reduction in water uptake reported here is considerable and desirable for development of improved protein plastic molding powders. Unfortunately, in this series of derivatives it is accompanied by brittleness and a concomitant loss of strength of molded objects. Comparatively few tensile strength measurements were made on the acylated caseins, but the following figures illustrate the loss in strength accompanying acylation. Test specimens molded from casein hardened with formaldehyde had a tensile strength of 7000-9000 pounds per square inch, whereas for a modified casein containing 8.8% propionyl which had also been treated with formaldehyde, the tensile strength was 4300 pounds. Figures for commercial casein plastics are reported as high as 10,000 pounds.

In conclusion, a few remarks may be made concerning the moldability of casein and our modified products. Commercial casein plastics are produced by mixing rennet casein with relatively large quantities of water to yield a powder containing 35% water, extruding, shaping, hardening, conditioning, and dip polishing. In direct compression molding, untreated rennet or acid casein containing 15 to 20% water flows well under moderate pressure; but the molded pieces, to be converted into durable finished articles, must be immersed in form-

aldehyde solutions for considerable periods. If casein is pre-hardened with formaldehyde, its flow is seriously impaired and at least 20% water must be used in compression molding; as a consequence, the molded articles shrink considerably. The flow of acylated casein prehardened with formaldehyde may be considered as intermediate between those of untreated casein and formaldehyde-hardened casein. Although acylated casein derivatives do not flow so well as untreated casein in the presence of 15 to 20% water, they flow much better than formaldehyde-hardened casein. This quantity of water represents the maximum amount which can be used to plasticize these derivatives because of their reduced affinity for water, so that the excellent flow characteristic of untreated casein containing larger amounts of water cannot be attained. However, the acylated materials, containing only 12% water, flow well enough to permit direct compression molding of simple pieces in a positive-type mold, and thus the finished articles neither require immersion in formaldehyde nor show excessive shrinkage. The brittleness of acetylated, propionylated, and butyrylated casein plastics is their outstanding weakness. It is possible that incorporation of suitable fillers, together with some hitherto unrecognized plasticizer other than water, will remedy this defect. Indications are that acylation with higher fatty acid residues such as palmitoyl, stearoyl, and oleoyl, results in superior products.

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